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645



Vol. 339 issue no. 6227

Soviet and French teams working through difficult winter conditions have completed a record of the effects of last year's Armenian earthquake. For the devastation and deaths caused, the 'quake was surprisingly small. Cover shows a surface fault with Spitak in the background. See page 675.

### HIS WEEK

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## psion exchanges

gmma rays from cold fusion? rere are two schools of thought. ge 667. And on page 690. a Sessment of calculated fusion les in isotopic hydrogen molules concludes that a 5 to 10enhancement of electron MSS is required to bring calcuated cold fusion rates up to the age of values claimed experientally. The authors "know of plausible mechanisms for hieving such enhancement".

#### Noney grows on trees

ne debate about the destruction tropical rainforests assumes ne question to be one of econversus ecology. But the due of products such as fruit ad latex, which do not involve eforestation, may be two to bree times higher than that of priper exports. Commentary. **b**ge 655.

#### Unase connections

link between cell-cycle control nd the regulation of transcripon is provided by the discovery rat the mouse kinase that phosporylates a repetitive domain of NA polymerase is encoded by the rouse homologue of cell-cycle introl gene cdc2. Page 679.

#### pper counts

the high-temperature superinductor Bi CaSr Cu O8, it is the oper oxide planes that contain e Fermi liquid' electronic ates central to superconductivin the BiO plane is non-metallic. ige 691.

#### itinction patterns

ircpe has far fewer tree species an eastern Asia, where the ther species diversity may be e to lower rates of glacial exttion. The role of factors such Hong periods of isolation may ess important than often supsed, page 699.

#### Coded messages

The magnetically disordered materials known as spin glasses constitute a model for a new form of error-correcting code in information transmission. Decoding a signal is equivalent to finding the ground state of the spin glass. and can result in uniquely low error probabilities, pages 693 and 662.

#### Altering enzymes

A moiecular model of the complex formed between tissue-type plasminogen activator (t-PA) and its physiological inhibitor, based on the known three-dimensional structure of a trypsin-inhibitor complex, has facilitated the engineering of an inhibitor-resistant t-PA. Pages 721 and 658.

#### Pacific atmospheres

Although industrial processes and the burning of fossil fuels have led to large increases in sulphur dioxide emissions in the Northern Hemisphere, over the



Pacific it is biologically produced compounds, mostly dimethyl sulphide from the ocean, that are the main influence on atmospheric sulphur levels and the climatic consequences. Page 685. Pacific nitrate levels, however, seem mainly dependent on anthropogenic nitrate production from continental America, page 687.

#### Guide to authors

Facing page 726.

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#### **BOOK REVIEWS**

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	Position	Sequence 5'-3'	Name	Region
	139-145	CTTATGGAGATTTGTTTCAGC	STP. 120	C.
	37-43	CGGCAAAAACAAATCAACAG	STP. 121	V <sub>72</sub>
	49-56	TGTCCTTGCAACCCCTACCC	STP. 073	· V <sub>y4</sub>
	35-42	TGTGCACTGGTACCAACTGA	STP. 094	V <sub>75</sub>
	55-62	(GGAA)TTCAAAAGAAAACATTGTCT	STP. 107	V <sub>76</sub>
	18-24	AAGCTAGAGGGTCCTCTGC	STP. 102	V <sub>7</sub> 6 V <sub>7</sub> 7
	158-165	CGAATTCCACAATCTTCTTG	STP. 110	$\vec{c_s}^7$
	79-86	(GGA)ATTCAGAAGGCAACAATGAAAG	STP. 111	
	65-72	TTCCTGGCTATTGCCTCTGAC	-	
	61-6 <b>8</b>	CCGCTTCTCTGTGAACTTCC	••	
	42-49	CAGATCCTTCCAGTTCATCC	•	
	72-78	TCAAGTCCATCAGCCTTGTC		
	18-25	CGCAGAGCTGCAGTGTAACT	STP. 076	
	65-72 61-68 42-49 72-78	TTCCTGGCTATTGCCTCTGAC CCGCTTCTCTGTGAACTTCC CAGATCCTTCCAGTTCATCC TCAAGTCCATCAGCCTTGTC	STP. 119 STP. 075 STP. 082 STP. 113	V <sub>81</sub> V <sub>83</sub> V <sub>84</sub> V <sub>85</sub> V <sub>86</sub> V <sub>87</sub>

The position of the nucleotide sequence of the primer is indicated by the corresponding amino-acid number counted from the putative N-terminal cleavage site in each reference. For the  $V_{86}$  primer, a sequence common to p $\lambda$ 12, Z53 and Z49 was chosen. The 3' 15 bases of this primer are also common M23 (ref. 4).

The amino-acid sequences deduced from the junctional nucleotide sequences indicate that IEL γδ TCR would have a high degree of structural diversity in the V-J junctional regions (data not shown); but diversity is not limited to these regions because the  $V_{\gamma 7}$ -coded  $\gamma$ -chain can pair with either the  $V_4$ ,  $V_5$ ,  $V_6$  or  $V_7$   $\delta$ -chain. This diversity of the IEL  $\gamma\delta$  TCR is reminiscent of that observed for the yo TCR expressed on the thymocytes of adult mice 18,19. The IEL yo TCR, however, clearly comprise a unique subset distinct from those on adult thymocytes which use  $V_{y4}$  and  $V_{85}$  gene segments predominantly.

The yo TCR expressed on DEC, the other known epitheliumassociated  $\gamma\delta$  T-cell subset, utilize a single  $V_{\gamma}$  ( $V_{\gamma5}$ ) and a single  $V_{\delta}$  ( $V_{\delta 1}$ ) gene segment and have no junctional diversity<sup>14</sup>. This suggests that the ligand for DEC yo TCR is monomorphic unlike those of  $\alpha\beta$  TCR<sup>14</sup>. By contrast, IEL certainly have the capacity to recognize structurally diverse ligands with their highly diverse γδ TCR. This, plus the fact that IEL are CD8-positive 20,21 strongly suggests that their ligand is composed of a structurally variable peptide presented by a class I or class I-like protein of the major histocompatibility complex (MHC). The high level of diversity concentrated in the V-(D)-J junctions is consistent with the recognition of variable peptides, if the folding of polypeptide chains is similar for TCR  $\gamma\delta$  and immunoglobulin molecules<sup>22</sup>. The origin of the postulated peptides is a matter of speculation. One possibility is that they originate from a relatively large set of self proteins whose syntheses are induced when the epithelial cells are under stress. Another possibility is that the peptides arise from viruses, bacteria and other microorganisms that are prone to infect the intestinal epithelium cells. The preferential usage of the  $V_{\gamma\gamma}$  segment may reflect its affinity for a limited number of class I or class I-like protein(s) that may be expressed on intestinal epithelial cells.

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FIG. 1

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Amemiya, Y. & Miyahara, J. Nature 336, 89-90 (1988). 29. Heitig, J. S. & Tonegawa, S. Nature 322, 838-840 (1986).

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## Cloning of murine $\alpha$ and $\beta$ retinoic acid-receptors and a novel receptor y predominantly expressed in skin-

Arthur Zelent, Andrée Krust, Martin Petkovich Philippe Kastner & Pierre Chambon®

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IN addition to having profound effects on embryonic pattern formation 1-5, retinoic acid (RA) has striking effects on differentiation and maintenance of epithelial cells in vivo and in vitro (reviewed in refs 6 and 7). Skin is a major target organ for retinoids both in its normal<sup>6-9</sup> and pathological states 10. The discovery of two human nuclear receptors for RA (hRARa and hRARB) acting as transcriptional RA-inducible enhancer factors 11-14 has provided a basis for understanding how RA controls gene expression 15.16 To investigate the specific role that RARs might play during development and in adult tissues, we have cloned the mouse RARa and RARβ (mRARα and mRARβ). Their amino-acid sequences are much more homologous to those of hRARα and hRARβ. respectively, than to each other, which suggests strongly that RAR α- and β-subtypes have different functions. Most interestingly we have discovered a novel RAR subtype (mRARy) whose expression in adult mouse seems to be highly restricted to skin, whereas RARa and RARB are expressed in a variety of adult tissues. Furthermore. both mRARa and mRARy RNAs are readily detected in undifferentiated F9 embryocarcinoma (EC) cells, whereas mRARB messenger RNA is induced at least 30-fold in RA-differentiated F9 cells.

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To whom correspondence should be addressed

A .... Here his Ser Asp. Ser See Ser Cya Pro Thr Pro Gly Gly Gly Sin Lee Ass. Gly Tyr Pro Val Pro Pro Tyr Ala Phe: Phe Pro Pro Met Lee Gly Gly Lee Ser Pro Pro Gly Ala Leu Thr Ser Leu Gin Ris Gin Leo Pro Val Ser Gly Tyz Ser Thz Pro Ser Pro Ala Thr 61 lie Giu Thr Gin Ser Ser Ser Ser Giu Giu tie Val Pro Ser Pro Pro Ser Pro Pro Pro Lou Pro Arg tie Tyr Lys Pro Cys Phe Val Cys Gin Asp Lys Ser Jer Jly Tyr His Tyr Bly Val Ser Ala Cys Glu Gly Cye Lys Gly Phe Phe Arg Arg Ser 11e Gln Lys Asn Het Val Tyr Thr Cys His Arg Asp Lys Asn Cys Lie Lie Asn Lys Val Thr Arg Asn Arg Cys 121 Gin Tyr Cys Arg Leu Gin Lys Cys Phe Asp Val Gly Net Ser Lys Glu Ser Val Arg Ass 141 Asp Arg Asn Lys Lys Lys Glu Ale Pro Lys Pro Glu Cys Ser Glu Ser Tyr thr Leu D The Pro Glu Val Gly Glu Leu Ile Glu Lys Val Arg Lys Als His Gln Glu The Phe Pr Ala Leu Cys Gin Leu Gly Lys Tyr Thr Thr Asn Asn Ser Ser Glu Gin Arg Vai Ser Le Asp Ile Asp Leu Trp Asp Lys Phe Ser Glu Leu Ser Thr Lys Cys Ile Ile Lys Thr Va Glu Phe Ala Lys Sin Leu Pro Siy Phe Thr Thr Leu Thr Ile Ala Asp Sin Ile Thr Le Leu Lys Ala Ale Cys Leu Asp fle Leu Ile Leu Arg fle Cys Thr Arg Tyr Thr Pro Ile 261 Gin Amp The Met The Phe See Amp Gly Leu The Leu Amn Arg The Gin Met Him Amn Ala E 281 Gly Phe Gly Pro Leu Thr Asp Leu Val Phe Ale Phe Ale Ash Gln Leu Leu Pro Leu 51 Het Amp Amp Ala Glu Thr Gly Leu Leu Ser Ala ile Cym Leu ile Cym Gly Amp Ard Gir 321 Asp Leu Glu Gln Pro Asp Lys Val Asp Met Leu Gln Slu Pro Leu Leu Glu Ala Leu Lys Arg Wal Tyr Wal Arg Lys Arg Arg Pro Ser Arg Pro His Met Phe Pro Lys Met Leu Met Lys tie Thr Asp Leu Arg Ser lie Ser Ala Lys Gly Ala Glu Arg Val lie Thr Leu Lys Met Siu lie Pro Siy Ser Het Pro Pro Leu lie Gin Siu Het Leu Siu Asn Ser Siu Siy Leu Asp Thr Leu Ser Sty Sin Ser Sty Sty Sty Thr Arq Asp Sty Sty Sey Leu Ala Pro Pro Sty 441 Pro Gly Ser Cys Ser Pro Ser Leu Ser Pro Ser Ser dis Arg Ser Ser Pro Ala Thr Tin Akn 461 Ser Pro End b Het Phe Asp Cys Het Asp Val Leu Ser Val Ser Pro Gly Gin Ite Leu Asp Phe Tyr Thr Ala Ser Pro Ser Ser Cys Met Leu Gln Glu Lys Ala Leu Lys Ala Cys Leu Ser Sly Phe Leu The Gin Ala Giu Tep Gin His Arg His The Ala Gin See Ile Giu The Gin Ger The See В Ser Glu Glu Leu Val Pro Ser Pro Pro Ser Pro Leu Pro Pro Pro Arg Val Tyr Lys Pro Cvs Phe Val Cvs Gln Asp Lys Ser Ser Gly Tyr His Tyr Gly Val Ser Ala C/s Glu Gl/ Cys Lys Gly Phe Phe Arg Arg Ser Ile Glo Lys Ann Net Ile Tyr Thr Cys His Arg Asp Lys Asn Cys Val Ile Asn Lys Val The Arg Asn Arg Cys Gln Tyr Cys Arg Leu Gin Lys 141 Cys Phe Glu Val Gly Met Ser Lys Glu Ser Val Arg Asn Asp Arg Asn Lys Lys Lys Glu Pro Ser Lys Gla Glu Eys Thr Glu Ser Tyr Glu Not Thr Ala Glu Leu Asp Asp Leu D The Glu Lys Ile Arg Lys Ale His Gla Glu The Phe Pro Ser Leu Cys Gla Leu Gly Lys Tyr Thr Thr Asn Ser Ser Ale Asp Bis Arg Val Arg Leu Asp Leu Gly Leu Trp Asp Lys Phe Ser Glu Leu Ala Thr Lys Cys Ile Ile Lys Ile Val Glu Phe Ala Lys Arg Leu Pro 221 Gly Phe The Gly Leu The Ile Ala Asp Gln Ile The Leu Leu Lys Ala Ala Cys Leu Asp lie Leu lie Leu Arg lie Cys Thr Arg Tyr Thr Pro Glu Gin Asp Thr Het Thr Phe Ser Asp Cly Leu Thr Leu Asn Arg Thr Gln Het Ris Asn Ala Gly Phe Gly Pro Leu Thr Asp Leu Val Phe Thr Phe Als Asm Gin Leu Leu Pro Leu Glu Met Asp Asp Thr Glu Thr Gly

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FIG. 1 Amino-acid sequences of mRAR $\alpha$  (a) and  $\beta$  (b), and nucleic-acid and seduced amino-acid sequences of mRAR $\gamma$  (c). Nucleotides and amino acids are numbered on the left side of each sequence. An in-frame termination codon (positions 145–147) in the 5 untranslated region of mRAR $\gamma$  cDNA is underlined in c. Regions A, C and E are boxed, and the location of regions A-F is indicated on the right side of each sequence (see text). Amino acids differing between mRAR $\alpha$  and mRAR $\beta$  and their human cognates are indicated in a and b under the murine sequences. The termination codon at the end of the mRAR $\gamma$  sequence is underlined in c and the ends of mRAR $\alpha$  and mRAR $\beta$  amino-acid sequences are indicated in a and b. The sequences of mRAR $\alpha$  and mRAR $\beta$  cDNAs are available upon request.

Leu Leu Ser Ala Ile Cys Leu Ile Cys Gly Asp Arq Gin Asp Leu Glu Glu Pro Thr Lys Vel Asp Lys Leu Gin Glu Pro Leu Leu Glu Ala Leu Lys Ile Tyr Ile Arq Lys Arq Arq Pro Ser Lys Pro His Met Phe Pro Lys Ile Leu Met Lys Ile Thr Asp Leu Arq Ser Ile

Ser Ala Lys Gly Ala Glu Arg Val Tie The Leu Lys Met Glu Tie Pro Gly Ser Met Pro

Pro Leu lie Gin Giu Met Leu Giu Aan Ser Giu Giy Maa Glu Pro Leu The Pro Ser Ser

421 Ser Gly Aan lie Ala Glu His Ser Pro Ser Val Ser Pro Ser Ser Val Glu Asn Ser Gly F

VETHODS. Approximately  $2\times10^6$  recombinant phage from an 11.5-day, randomly-primed, mouse-embryo  $\lambda$ gt10 cDNA library (donated by B. Galliot and D. Duboule) were screened with purified hRAR $\alpha$  and hRAR $\beta$  cDNA probes derived from clones hRAR $\alpha$ 0 and hRAR $\beta$ 0.11.13 and  $^{32}$ P-labelled by random priming  $^{24}$  to a specific activity of  $10^9$  c.p.m.  $\mu$ g $^{-1}$  of DNA. Plaque lifts onto introcellulose filters and treatment of the filters before hybridization were sarried out as described previously  $^{24}$  Hybridization was carried out at 37 °C 10 r  $^{-2}$ 4 h in 5  $\times$  SSPE (0.75 M NaCl. 50 mM NaH $_2$ PO $_4$  and 5 mM EDTA, pH 7.4). 40% formamide, 0.2 mg mi $^{-1}$  sheared and denatured salmon-sperm DNA.

and 1  $\times$  Denhardt's reagent<sup>24</sup>. The most stringent wash was done in 2  $\times$  SSPE plus 0.1% SDS/0.06% sodium pyrophosphate (NaPPi) at 50 °C for 20 min. Filters were exposed for 36 h at -80 °C (one intensifying screen, Kodak XRO-5 film). Positive clones were rescreened under the same conditions and phage DNA was prepared from the clones which remained positive. These clones were divided into three groups based on Southern blot hybridization data with hRARa and hRARB cDNA probes and restriction enzyme digest patterns. Selected cDNAs from each group were subcloned into either pTZ19R, or pEMBL18 and 19 vectors; single stranded DNA was prepared<sup>24</sup> and sequenced using the dideoxy chain-termination procedure<sup>25</sup>. Two clones sequenced either on both strands (mRARy, sequence shown in panel c), or on one strand (mRARB, panel b; any ambiguity was resolved by sequencing on the other strand). The mRAR $\alpha$  sequence was derived from two clones which were sequenced on both strands and overlapped between amino acids 64 and 283 of the sequence displayed in panel a. A mRARlpha cDNA clone containing the entire open reading frame was subsequently constructed in pSG5 (ref. 21) by using the unique Bsu36I site located in the DNA sequence encoding amino acids 182-184.

44) Val Ser Gin Ser Pro Leu Leu Gin End Val

An 11.5-day-old total mouse embryo Agt10 complementary DNA library was screened with hRARa and hRARB cDNA probes: 81 clones were isolated, of which two sets were identified as mRARα and mRARβ on the basis of a 98% homology of their cDNA-deduced amino-acid sequence with that of hRARa and hRARB, respectively (Fig. 1a, b). Less homology with RARa or RARB was found for a third set of clones, although the deduced amino-acid sequence (Fig. 1c) was obviously related to both of them (Fig. 2; the A-F regions in Figs 1 and 2 were as previously defined in refs 11, 13 and 16). This new member of the mouse RAR subfamily was designated mRARy. The greatest amino-acid sequence similarities among the three mRARs were found in the regions corresponding to the DNAbinding domain (region C, 95%) and the ligand-binding domain (region E, 85% identity between mRARa and mRARy, and 90% identity between mRARB and either mRARa or y), suggesting that mRARy recognizes the same responsive element and binds the same ligand as  $mRAR\alpha$  and  $mRAR\beta$  (see below). Region B is also conserved (75%, 86% and 79% identity between mRARy and mRARa, mRARy and mRARB, and mRARa and mRARB, respectively), whereas no conservation was seen in this region when comparing nuclear receptors that bind different ligands (ref. 16 and refs therein). The D region, which is not conserved across the nuclear-receptor family of a given species and may act as a hinge region 16, is less conserved among mRARs. Both the N- and C-terminal segments of region D, however, are highly conserved, although the central segment is not (hatched box in Fig. 2). No significant similarity was found between mRARs in region A (encoded in an exon different from that encoding region B, see ref. 13), nor in region F, both of which also vary within a given species between the different nuclear receptors 16.

By contrast, there is an almost complete conservation of amino-acid sequence between the A regions of hRARa and mRARa (98%), and hRAR $\beta^{13,14}$  and mRAR $\beta$  (94%), and between the corresponding F regions (90% for hRARa and mRARa, and 92% for hRARB and mRARB). Similarly, the entire D region of a given RAR subtype is conserved across

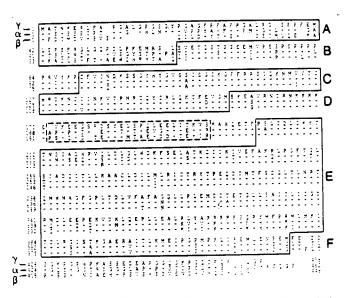
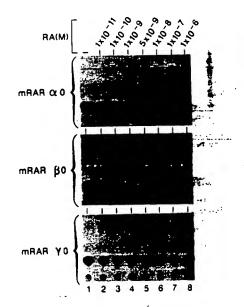


FIG. 2 Amino-acid alignment of the mRAR $\alpha$ , mRAR $\beta$  and mRAR $\gamma$  (as indicated). The single letter amino-acid code is used, and the number of the last amino acid in each sequence is given at the end of the alignment. Numbers on the left side correspond to the first amino lack the alignment. Regions A. C and E are boxed and regions A- $\beta$  are do. If along with capital letters on the right side of the figure. The non-conserved part within region D is boxed with a hatched line. Gaps have been intribuced to cotain the optimal alignment of the mRAR $\gamma$  sequence with that of mRAR $\alpha$  and mRAR $\beta$  Dashes represent mRAR $\alpha$  and mRAR $\beta$  amino acids which are identical with those of mRAR $\gamma$ .

species (98% identity between both hRARa and mRARa, and hRARB and mRARB). This high degree of conservation of the A, D and F regions for a given RAR subtype contrast, with the lack of or lower conservation of the same region, among the various RARs in a given species (see above) and also with the lower conservation of regions A/B, D and F for a given steroid-hormone receptor across species 16,17. Thus the A, B, D and F regions may have specific functions not performed by the C and E regions, but necessary for the three RARs to exert their specific physiological roles. Note that the A/B regions of the oestrogen and progesterone receptors have been implicated in specific transcriptional transactivation of some target genes 16,18-20.

When mRARα, mRARβ and mRARγ cDNAs were expressed<sup>21</sup> in HeLa cells together with a reporter plasmid (TRE3)<sub>3</sub>-tk-CAT containing a RA-responsive element (Fig. 3), all three receptors responded similarly to all-trans RA. As was the case for hRARα and hRARβ<sup>11,13</sup>, retinol was much less efficient at the same concentrations (data not shown). No obvious difference was observed between the dose responsiveness of mRARα and mRARβ, in contrast with results obtained previously with human chimaeric RARα and RARβ<sup>13</sup>. This may be due to the use of a less sensitive responsive element in the present study.



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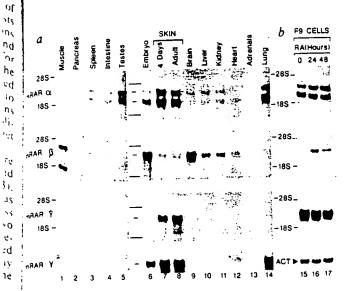
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FIG. 3 RA-dependent transcriptional trans-activation by mRARs transiently expressed in HeLa cells. HeLa cells were co-transfected as described with pSG5-based expression vectors containing the entire cDNA of either mRAR $\alpha$  (mRAR $\alpha$ 0), mRAR $\beta$  (mRAR $\beta$ 0) or mRAR $\gamma$  (mRAR $\gamma$ 0), and a reporter plasmid. (TRE3)3-tk-CAT. carrying a synthetic RA-responsive element (RARE). After transfection (24 h), cells were fed with media containing increasing concentrations of RA as indicated (0–1  $\times$  10<sup>-6</sup> M, lanes 1–8, respectively) and collected 48 h after transfection for determination of CAT (chloramphenicol acetyltransferase) activity.

METHODS. HeLa cells (~10<sup>6</sup> per dish) were co-transfected with 0.5 μg of a given mRAR expression vector, 2 μg of reporter plasmid and 2 μg of pCH110 (Pharmacia, a β-galactosidase expression vector used as an internal control to normalize for variations in transfection efficiency). The total amount of transfected DNA was adjusted to 20 μg by addition of carrier DNA (BSM13+). Cell culture media, treatment of cells, preparation of cytosolic extracts, and CAT assays were carried out as previously described 1:13. The reporter plasmid (TRE3)<sub>3</sub>-tk-CAT (ref. 26) contains a trimer of a synthetic RARE (5'-AGCITAGGTCAGGGACGTGACCTT-3') inserted by inserting the reconstructed mRARα cDNA (see Fig. 1) into the *Eco*RI site of pSG5 (ref. 21), mRARβO was obtained by inserting the *Eag1 BamHI* fragment of mRAPβ cDNA into the *BamHI* site of pSG5 with the help of a *BamHI* (*Eag1* adaptor, and mRARγO was constructed by ligating the *Eco*RI flacked mRARγ cDNA (Fig. 1c) into the *Eco*RI site of oSG5.



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The expression of mRARs was investigated using specific oligonucleotide (mRARa and mRARy) or randomly primed cDNA (mRARβ and mRARγ) probes (Fig. 4a). Two mRARα RNAs (~3.8 kilobase (kb) and 2.8 kb) were found in all mouse tissues including skin, and in 11.5-day old embryo (see legend to Fig. 4a for pancreas and adrenal). Compared with mRARa RNA, the 3.4-kb mRARB RNA was relatively more abundant in brain and in total 11.5-day embryo, and lower in skin and lung, than in other tissues. No 3.4-kb mRARB RNA could be detected in spleen, intestine and testis, and an additional 1.9-kb species was found only in muscle (due to the use of different probes and exposure conditions, the mRAR3 signal was amplified at least 20-fold relative to the mRARa signal in Fig. 4a). By contrast, it is remarkable that mRARy RNA was detected at levels at least as high as those of  $mRAR\alpha$  only in the skin of both 4-day-old and adult animals (upper and third row in Fig. 4a). Using a probe of higher specific activity, mRARy RNA was detectable in total 11.5-day embryo and in lung, and at trace levels in spleen (lower row in Fig. 4a). mRARa and mRARy RNAs were also present in F9 EC cells which differentiate to endodermal-like cells upon exposure to RA<sup>22</sup> (Fig. 4b). mRARB RNA was induced by RA (at least 30-fold from densitometry), whereas no variation was seen for mRARa RNA, and mRARy RNA decreased by two-fold. A similar induction of mRARB RNA has been observed by L. Gudas et al. (personal communication). Whether this induction is transcriptional, as for hRARB in a human cell line23, and is mediated by mRARa

or mRARy or both, is unknown. In summary, three RAR subtypes are expressed in the mouse, two of them being strikingly homologous to human R NR and RARB. That hRARa and mRARa, and hRARB and mRARB are more homologous to each other than either hR VR-c and hRARB, or mRARa and mRARB, strongly suggests that the three RAR subtypes exert specific functions perhaps by resultiing the transcription of different genes at different times of development and in specific cells. In this respect, it is noteworthy that mRARy expression seems to be highly restricted to skin which is known to be an exquisite RA target in both normal and pathological states whether mRARy is specifically

FIG. 4 Northern blot analysis of mRARα, mRARβ and mRARγ poly(A)\* RNA from various mouse tissues (as indicated in a lanes 1-14), and F9 EC cells (b. lanes 15–17) before (lane 15), and after 24 h (lane 16) and 48 h (lane 17) treatment with RA (3.3  $\times$  10<sup>-7</sup> M). Poly(A)\* RNA (4  $\mu$ g) was loaded in all lanes. The 28S and 18S rRNA standards were taken as being 4,712 (ref. 28) and 1.869 (ref. 29) bases long, respectively. a, mRAR $\alpha$  and mRAR $\gamma$ sequences were detected with specific [32P]-end labelled oligonucleotide probes. The entire mRARB cDNA labelled with [32P] by random priming was used to detect mRARB RNA. Hybridizations were performed using the same blots, first hybridized with the mRARa probe (exposure time, 7 days at -80 °C; two intensifying screens and Kodak XRO-5 film), then with the mRARB probe and finally with the mRARy probe (exposure times, 4 days). The lower panel corresponds to a different blot hybridized only with a randomly primed entire mRARy cDNA probe (exposure time, 24 h). Actin RNA could be revealed with a cytoskeletal actin cDNA probe30 in all cases except for pancreas and adrenal preparations suggesting RNA degradation (data not shown). b. Three blots were hybridized with randomly primed  $[^{32}P]$ -labelled entire mRARlpha, mRAReta and mRAR $\gamma$  cDNA probes (exposure time, 12 h). The filters were also probed with the actin cDNA probe (lower panel, ACT)

METHODS. RNA was extracted using the GnSCN-CsCl procedure 31, Poly(A) RNA<sup>32</sup> was electrophoresed on 1% agarose-1.1 M formaldehyde gels<sup>33</sup> and transferred to nitrocellulose filters<sup>24</sup>. Hybridisation was as described in the legend to Fig. 1, except that 50% formamide was used and hybridisation was at 42-45 °C (37-40 °C for oligonucleotide probes) for 18 h. Filters were dehybridized by 5-min treatments in 0.05 × SSPE at 90 °C. Specific activity of all randomly-primed cDNA probes and labelled oligonucleotides was ~109 and 108 c.p.m.  $\mu g^{-1}$  DNA, respectively. The most stringent wash (15 min) was at 65 °C in 0.1 × SSPE plus 1.0% SDS/0.03% NaPPi for filters hybridized with randomly-primed cDNA probes and at 55 °C in 1 × SSPE plus 1.0% SDS/0.03% NaPPi with [32P]-lacelled oligonucleotide probes.

expressed in keratinocytes remains to be seen. Finally, the numerous effects of RA on development and the presence of the three RARs in mouse embryo and differentiated F9 cells, raises the question as to whether they exhibit specific patterns. of expression and function during embryogenesis.

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